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Comparative Genomic Hybridization Reveals a Specific Pattern of Chromosomal Gains and Losses During the Genesis of Colorectal Tumors

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Comparative genomic hybridization was used to screen the DNA extracted from histologically defined tissue sections from consecutive stages of colorectal carcinogenesis for chromosomal aberrations. No aberrations were detected in normal epithelium (n = 14). Gain of chromosome 7 occurred as a single event in low-grade adenomas (n = 14). In high-grade adenomas (n = 12), an overrepresentation of chromosomes 7 and 20 was present in 30% of the cases analyzed. The transition to colon carcinomas (n = 16) was characterized by the emergence of multiple chromosomal aberrations. Chromosomes 1, 13, and 20 and chromosome arms 7p and 8q were frequently gained, whereas chromosome 4 and chromosome arms 8p and 18q were recurrently underrepresented. The same tissue sections that were used for CGH were analyzed by means of DNA-ploidy measurements and immunohistochemical staining to quantify proliferative activity and p21/WAF-1 and TP53 expression. We observed that crude aneuploidy and increased proliferative activity are early events in colorectal carcinogenesis, followed by TP53 overexpression and the acquisition of recurrent chromosomal gains and losses during the progression from high-grade adenomas to invasive carcinomas. *Genes Chromosom Cancer 15:234–245 (1996).* © 1996 Wiley-Liss, Inc.*

INTRODUCTION

A series of genetic aberrations is required for tumor initiation and progression (Foulds, 1958; Nowell, 1976). Regarding colorectal carcinogenesis, these aberrations include the activation of the *SRC* and *RAS* oncogenes (Bolen et al., 1987; Bos et al., 1987) along with the inactivation of the *FAP* (Groden et al., 1991; Kinzler et al., 1991) and *DCC* (Fearon et al., 1990) tumor suppressor genes and loss of function of *TP53* (Baker et al., 1989). Those mutations occur sequentially. However, the accumulation rather than the sequence of aberrations seems to define the threshold towards malignant transformation (Fearon and Vogelstein, 1990). In addition to the mutation of certain genes, one of the early markers in colorectal carcinogenesis is the occurrence of crude aneuploidy as measured by DNA image cytometry (Steinbeck et al., 1993, 1994; Auer et al., 1994). This aneuploidization reflects a genomic instability, which might be a requirement for additional mutations to occur. On the chromosomal level, genetic instability is reflected by the acquisition of numerical and structural chromosomal aberrations that have been described both for adenomas and carcinomas from studies using chromosome banding analysis (Bardi et al., 1992, 1993, 1995; Muleris et al., 1994) and interphase cytogenetics (Herbergs et al., 1994).

To establish a microscopic phenotype/genotype correlation of defined stages of colorectal carcinogenesis, we used comparative genomic hybridization (CGH) to screen the DNA extracted from microdissected areas of formalin-fixed, paraffin-embedded tissue sections for chromosomal imbalances. CGH is based on a dual-color fluorescence in situ hybridization (FISH) using differentially labeled tumor and reference DNA as probes (Kallioniemi et al., 1992; Du Manoir et al., 1993). Copy number changes in the tumor genome are reflected in changes of the ratio of the two fluorochromes involved. One important feature of the technique is that formalin-fixed material can be analyzed as well (Speicher et al., 1993, 1995). This allows us 1) to correlate the findings with the clinical course by retrospectively analyzing archived material and 2) to perform additional investigations on the same tumor material, e.g., histology, studies for loss of heterozygosity (LOH), and the detection of protein expression using immunohistochemical methods (Ried et al., 1995; Heselmeyer et al., 1996).

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This paper is dedicated to Torbjörn Caspersson on the occasion of his 85th birthday.

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In this study we have attempted to generate a comprehensive picture of genomic and chromosomal aberrations during defined stages of colorectal carcinogenesis. A combination of CGH, DNA-ploidy measurements, detection of proliferation markers, and expression of tumor suppressor genes was applied to analyze normal epithelium, low- and high-grade adenomas, and invasive carcinomas of the colon. The results show that CGH is a well suited experimental approach for visualizing chromosomal gains and losses in histologically characterized tissue sections during solid tumor progression.

MATERIALS AND METHODS

Tissue Samples

The tumor material was collected between 1991 and 1994 from surgically removed tumors and diagnosed on hematoxylin-eosin (HE)-stained tissue sections at the Institute of Pathology, Flensburg, Germany, according to the WHO classification (1972). The clinical data are summarized in Table 1. Eight sections were prepared from each tumor and were used for histological diagnosis, immunohistochemistry (thickness 4 μm), DNA-ploidy measurements (8 μm), and microdissection (50 μm). A second HE-stained section was prepared after the sections for CGH analysis were cut, and the histological diagnosis was confirmed in all cases. All data were obtained from the dissected areas.

DNA Cytometry

Image cytometry was performed on Feulgen-stained histological sections to investigate the nuclear DNA content. The staining procedure, internal standardization, and tumor cell selection were based on methods described previously (Auer et al., 1994). All DNA values were expressed in relation to the corresponding staining controls, which were given the value 2c, denoting the normal diploid DNA content. All DNA values were presented in such relative units. The specimens were divided into two main groups: 1) diploid cases with a distinct peak in the normal 2c region and no cells exceeding 5c and 2) aneuploid cases with a main peak around the 4c region and varying numbers of cells (>5%) exceeding 5c. Examples of the DNA histograms are presented in Figure 1D,I.

Immunohistochemistry

The monoclonal antibody MIB-1 (Immunotech S.A., Marseille, France) was used for the detection of the Ki-67 antigen. The antibody discriminates nonproliferating (G_0) cells from proliferating (G_1-S-G_2-M) cells. Four-micrometer-thick tissue sections were prepared from formalin-fixed, paraffin-embedded specimens. The sections were dewaxed, rehydrated, and microwave treated in 0.01 M sodium citrate buffer for 2 × 5 min at 750 W. Endogenous peroxidase activity was blocked by immersion of the slides in 0.5% hydrogen peroxide in distilled water for 20 min; nonspecific staining was blocked by incubating sections in 1% bovine serum albumin (BSA) in Tris-buffered saline for 45 min. After incubation with the MIB-1 antibody (1:150 in 1% BSA) overnight at 4°C, the sections were washed in Tris-buffered saline and processed according to the routine avidin-biotin-peroxidase complex technique. Biotinylated antimouse affinity-purified IgG (Vector, Burlingame, CA; diluted 1:200 in Tris-buffered saline) was applied for 30 min, followed by incubation with avidin-biotin-peroxidase complex (Vectastain Elite; Vector). Diaminobenzidine was used as a chromogen. After counterstaining with hematoxylin, the slides were dehydrated and mounted. Any distinct nuclear MIB-1 staining was recorded as positive.

TP53 expression was detected using the DO-1 antibody (Santa Cruz; diluted 1:100 in 1% BSA), and p21/WAF-1 expression was detected with the WAF-1 antibody (Oncogene Sciences; diluted 1:15 in 1% BSA) following the procedure described above for the MIB-1 antibody. Examples of the staining patterns and a quantification of the results are shown in Figure 1 and Table 2.

CGH

Normal control DNA was prepared from peripheral blood lymphocytes of a cytogenetically normal male. Formalin-fixed and paraffin-embedded tumor samples were provided in 50-μm-thick tissue sections. The tissue was cut into small pieces and incubated in xylene (45°C for 15 min), followed by one wash in ethanol. After centrifugation, the samples were dried and resuspended in 1 ml NaSCN (1 M) and incubated at 37°C overnight. The tissue was washed and resuspended in 400 μl of DNA isolation buffer (75 mM NaCl, 25 mM EDTA, 0.5% Tween). Proteinase K was added to a final concentration of 1 mg/ml, and the tissue was incubated overnight at 55°C. The DNA was purified by phenol extraction. Normal male DNA was labeled in a standard nick-translation reaction substituting dTTP with digoxigenin dUTP (Boehringer Mannheim, Indianapolis, IN). Tumor DNA was labeled by substituting dTTP with biotin-16-dUTP (Boeh-

TABLE 1. Clinical Data and Histology of the Colon Tumors

Case No.	Age (years)	Sex	Histology[a]	Localization	Size (mm)
Low-grade adenoma					
105A	60	M	t-v	Sigmoid	8
106A	72	M	t-v	Sigmoid	32
107A	53	M	t-v	Sigmoid	10
108A	44	F	t-v	Sigmoid	10
109A	54	M	t	Rectum	6
110A	68	F	t-v	Rectum	15
111A	46	M	t	Rectum	15
113A	55	F	t	Rectum	15
115A	50	M	t-v	Sigmoid	12
119A	45	M	t-v	Rectum	15
120A	42	F	t	Sigmoid	5
121A	60	M	t-v	Rectum	10
123A	82	F	t-v	Rectum	25
124A	55	M	t-v	Sigmoid	15
High-grade adenoma					
9	89	M	t-v	Rectum	13
12	37	F	t	Rectum	5
13	63	F	t-v	Bauhin valve	25
15	50	M	t-v	Col. desc.	15
16	76	F	t	Sigmoid	12
18	67	F	t-v	Sigmoid	15
25	80	F	t-v	Rectum	5
29	74	F	t-v	Col. desc.	24
112	67	M	t	Col. desc.	10
114	71	F	t-v	Sigmoid	12
116	62	F	t-v	Sigmoid	12
122	74	M	v	Rectum	48
Carcinoma					
36	67	M	pT3, pN2, M0	Col. asc.	50 × 60
38	59	M	pT3, pN2, M1	Col. asc.	37
39	62	M	pT3, pN1, M0	Col. transv	40
41	61	M	n.d.	Col. asc.	38
42	79	F	pT3, pN2, M0	Rectum	55
43	81	F	pT3, pN1, M0	Rectum	45
44	72	M	pT3, pN2, M0	Rectum	55
46	87	F	pT3, pN0, M0	Sigmoid	60 × 40
47	69	M	pT3, pN0, M0	Sigmoid	55
50	70	M	n.d.	Rectum	60
51	52	F	pT3, pN2, M0	Sigmoid	40
65	78	M	pT3, pN1, M0	Sigmoid	90 × 50
68	62	F	pT3, pN1, M0	Rectum	40
86	54	F	pT4, pN2, M0	Rectum	7 × 45
88	73	F	pT3, pN0, M0	Sigmoid	70
89	88	F	pT3, pN2, M0	Sigmoid	30

[a]t-v, tubulovillous adenoma; t, tubular adenoma; v, villous adenoma; n.d., not determined.

ringer Mannheim). Labeling with biotin-16-dUTP was performed using a random primed labeling kit (Boehringer Mannheim) when only small amounts of tumor DNA (200–500 ng) could be recovered from the tissue sections.

For CGH, 200 ng of normal, digoxigenin-labeled DNA and 200 ng of biotin-labeled tumor DNA were ethanol precipitated in the presence of 10 μg of salmon sperm DNA and 30 μg of the Cot-1 fraction of human DNA (Gibco BRL, Gaithersburg, MD). The probe mixture was dried and resuspended in 10 μl hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate). The DNA was denatured at 76°C for 5 min and allowed to preanneal at 37°C for 30 min. The metaphase preparations were denatured at 80°C for 2 min in 70%

deionized formamide, 2× SSC, and were dehydrated through an ethanol series (70%, 90%, 100%). The probe mixture was applied to the denatured metaphase chromosomes under a coverslip (18 mm^2), sealed with rubber cement, and hybridized for 4 days at 37°C.

Posthybridization steps were performed as described in detail elsewhere (Ried et al., 1992). The biotinylated sequences were detected with fluorescein conjugated to avidin (Vector). Probe sequences haptenized with digoxigenin were visualized with antidigoxigenin Fab fragments conjugated to rhodamine (Boehringer Mannheim). Chromosomes were counterstained with DAPI and embedded in an antifading agent to reduce photobleaching.

Microscopy and Digital Image Analysis

Gray-level images were acquired with a cooled charge coupled device (CCD) camera (Photometrics, Tucson, AZ) mounted on a Leica DMRBE epifluorescence microscope using filters specific for DAPI, fluorescein, and rhodamine (TR1, TR2, and TR3; Chroma Technologies, Brattleboro, VT). Chromosomes were identified using DAPI banding. Fluorescence ratio images were calculated as described, and the ratio profiles of individual reference chromosomes were determined by a custom-written computer program (Du Manoir et al., 1995) and were run on a Macintosh Quadra 950. Briefly, after the determination of the chromosomal axis for each chromosome in every metaphase, individual fluorescein and rhodamine profiles were calculated. These were used for the computation of the fluorescein/rhodamine average ratio profiles. The three vertical lines on the right side of the chromosome ideogram in Figure 2 represent different fluorescence ratio values between the tumor DNA and the normal DNA. The values are 0.75, 1, and 1.25 from left to right. These values were used as a threshold for under- or overrepresentation of chromosomal subregions in the tumor. The curves show the ratio profiles that were computed as mean values of ten metaphase spreads (Fig. 2). Regions rich in heterochromatin, e.g., on chromosomes 1, 13, 14, 16, and 22, cannot be evaluated because an excess of unlabeled Cot1 DNA in the hybridization solution prevents the calculation of ratio profiles in these regions.

RESULTS

CGH was used to map DNA copy number aberrations that occur at specific stages of colorectal carcinogenesis. The DNA was extracted from defined regions of normal mucosa (n = 14), low-grade adenomas (n = 14), high-grade adenomas (n = 12), and carcinomas (n = 16). The clinical data for all cases are summarized in Table 1. In addition to the delineation of specific chromosomal aberrations, we determined crude genetic instability by DNA-ploidy measurements. The expression levels of the *TP53* tumor suppressor genes and p21/WAF-1 as well as the proliferative activity were investigated immunohistochemically on the tissue sections used for CGH. The results of the different staining procedures and CGH experiments are presented as examples for an adenoma (case 112) and a carcinoma (case 88) in Figure 1. An example of an average ratio profile (case 112) that was used for the delineation of genetic changes in all cases is shown in Figure 2. The summary of the results is presented in Table 2.

Normal Mucosa

DNA-ploidy measurements revealed diploid values (2c) in normal epithelium. The proliferative activity was low (<5%), and TP53 and p21/WAF-1 expression could not be observed. No chromosomal aberrations were detected in this series of normal epithelium. The values for the chromosomes X and Y were consistent with the gender of the patients in all cases (data not shown).

Low-Grade Adenomas

Crude aneuploidy was detected in all samples analyzed. None of the 14 cases analyzed revealed a diploid DNA content. The DNA indices ranged from 2.6c (case 119A) to 3.9c (case 106A), indicating that DNA aneuploidy occurs as an early genetic alteration in low-grade adenomas. The proliferative activity increased to values of 30–70% of the cells analyzed. In a few cases, a moderate TP53 immunoreactivity was detected. However, p21/WAF-1 immunoreactivity was slightly increased in virtually all samples. No recurrent chromosomal aberrations were observed. In individual, single cases, a gain of chromosome 7 and 9p and a loss of chromosomal bands 5q21-23 were present (Fig. 3).

High-Grade Adenomas

All of the high-grade adenomas displayed crude aneuploidy, reflected in DNA indices from 2.6c (case 13) to 5.1c (case 9). The proliferative activity increased to values of approximately 70–80% of the cells. Moderately increased *TP53* immunoreactivity was now detected in 80% of the samples analyzed. p21/WAF-1 activity remained at levels that were observed in low-grade adenomas (Table 2). However, distinct chromosomal copy number

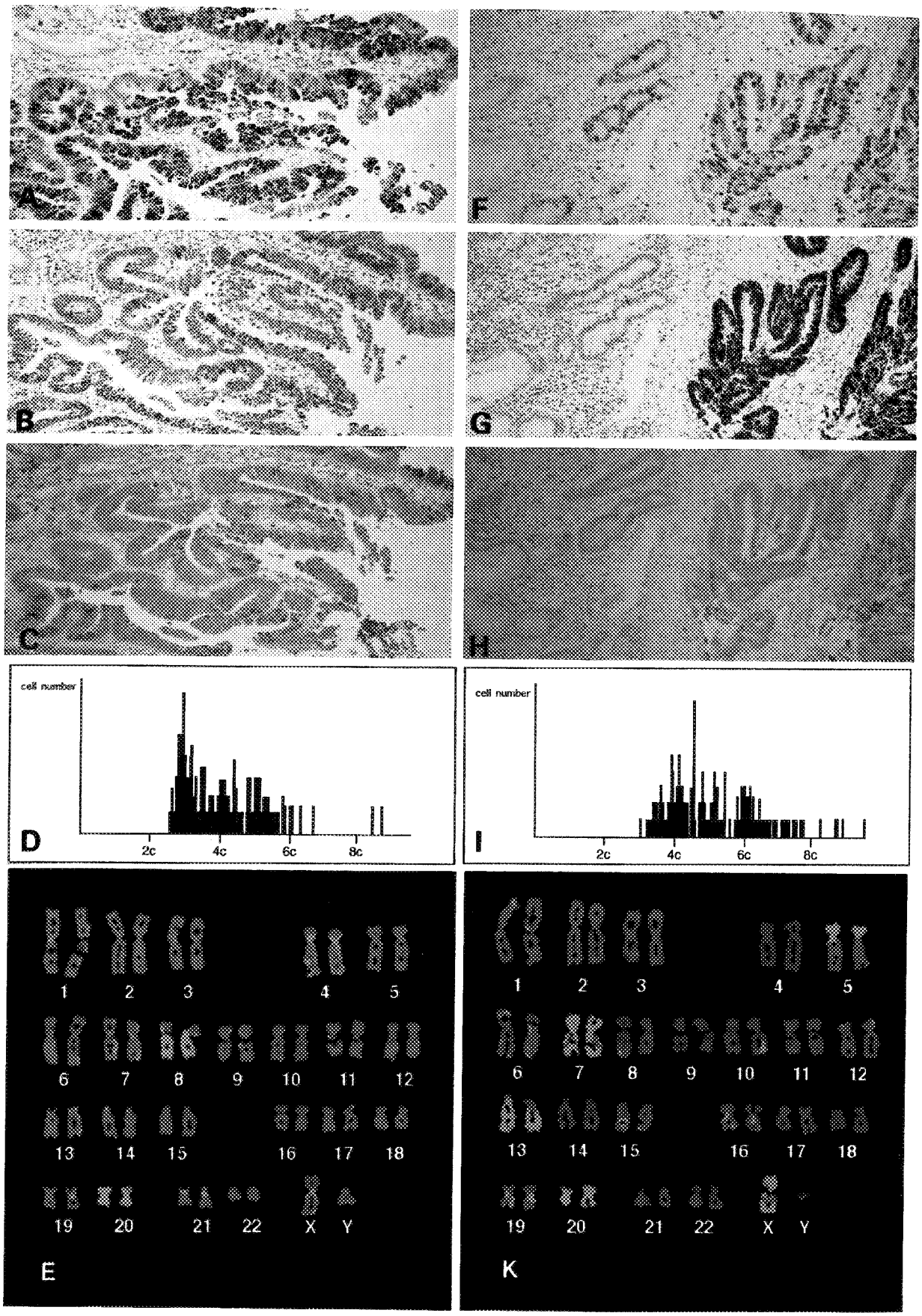
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Fig. 1.

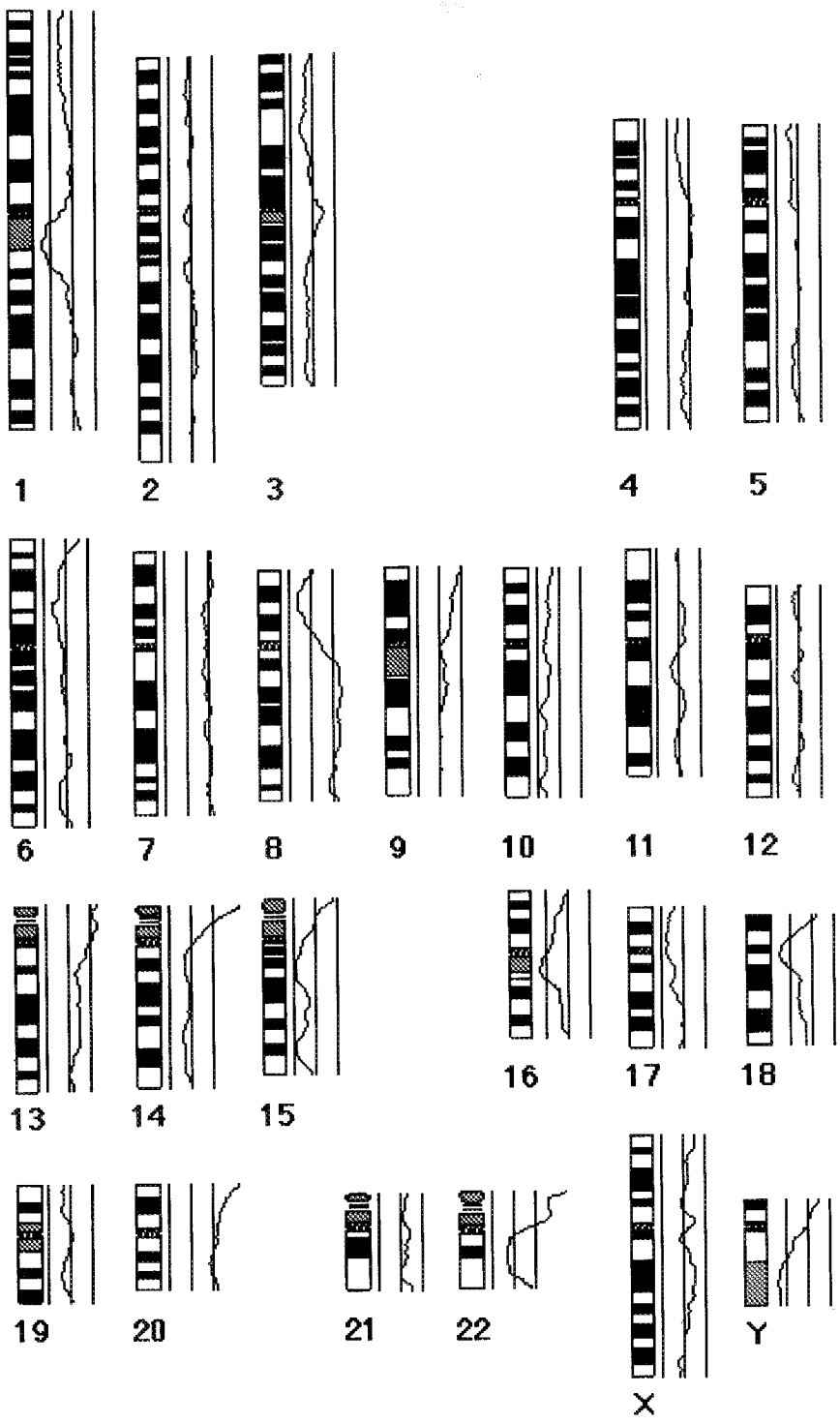

Figure 2. Example of an average ratio profile for a high-grade adenoma (case 112). The three vertical lines on the right side of the ideograms reflect different values of the fluorescence ratio between the tumor and the normal DNA. The values are 0.75, 1, and 1.25 from left to right. The ratio profile (curve) was computed as a mean value of ten metaphase spreads. Copy number changes are present on chromosomes 7 and 20 and on chromosome arms 4q and 8q.

Figure 1. Pictorial presentation of the immunocytochemical and genetic analysis of a high-grade colon adenoma (**A–E**, case 112) and a colon carcinoma (**F–K**, case 88). Staining patterns from using the antibody MIB-1 directed against the Ki-67 antigen (A,F), DO-1 directed against TP53 (B,G), and an antibody against p21/WAF-1 (C,H). DNA histograms obtained from image cytometry after Feulgen staining are shown in D and I. The result of the CGH analysis are presented in E and K. Blue color reflects balanced copy numbers between the reference and the tumor genome; red indicates a loss and green a gain in the tumor. Note the strong staining with the antibody against TP53 in G, along with a negative staining for p21/WAF-1. The degree of aneuploidy as well as the number of chromosomal aberrations is higher in the carcinoma. For a summary and a quantification of the results, see Table 2.

changes could be mapped: Gains included an overrepresentation of chromosome 7 and 20 material in four of 12 cases (30%). Losses were mapped to chromosome 18 and to 17p in two of 12 samples. In one instance, a regional high-level copy numberincrease could be mapped to chromosomal band 2p21. Examples of the different immunohistochemical staining patterns, the DNA histogram, and the CGH experiment are presented in Figure 1 for case 112. Figure 3 displays the karyogram of

TABLE 2. Summary of the Data on MIB-1, DO-1, and p21/WAF1 Staining, DNA-Ploidy Measurements, and CGH for All Cases of Low-Grade Adenomas, High-Grade Adenomas, and Carcinomas

Case No.	MIB (Ki-67)	DO-1 (p53)	WAF1/ P21	DNA	CGH
Low-grade adenoma					
105A	40	neg	10	2.9c	—
106A	30	neg	neg	3.9c	—
107A	60	neg	20	3.8c	—
108A	40	neg	neg	3.2c	—
109A	30	neg	neg	3.8c	—
110A	50	neg	neg	3.7c	+7
111A	60	neg	neg	2.5c	—
113A	20	neg	neg	2.9c	—
115A	40	neg	neg	2.7c	—
119A	60	neg	neg	2.6c	−5q
120A	70	neg	neg	3.0c	—
121A	40	neg	neg	2.9c	—
123A	40	<2	<2	3.0c	+9p
124A	60	neg	neg	3.1c	—
High-grade adenoma					
9	30	30	10	5.1c	+8q, +20
12	60	<5	2	4.1c	—
13	30	neg	5	2.6c	—
15	70	<2	<2	3.84c	+7, −17, −19, −22
16	70	5	10	2.8c	—
18	20,50	<2	<2	3.0c	—
25	80	<2	<2	2.8c	+2p, −10q, −17p
29	60	<2	<2	3.24c	—
112	70	15	<2	3.8c	+4q, +7, +8q, +20
114	70	neg	10	4.2c	+20, −11q, −18q
116	30	5	5	3.4c	+7p, +13q, +20
122	40	5	0	3.2c	+7, +9, +12, −18
Carcinoma					
36	90	neg	neg	n.d.	—
38	60	70	neg	4.1c	+1q, +5p, +6, +7p, +12p, +13q, +20, −8p, −18q
39	70	neg	neg	2.8c	----
41	20	5	<2	3.8c	+7, +8q, +12q, +13q, +17p, +20q, −8p
42	60	neg	20	3.4c	+3q, +5p, +7, +8q, +13q, +20q, −8p, −18
43	70	40	neg	6.8c	+1q, +7, +8q, +13q, +20, −4, −8p−17p
44	80	80	neg	4.8c	+8q, +14q, +17q, +18q, +20, −2q, −4, −8p, −9
46	60	20	15	4.8c	+13q, +Xp, −18
47	80	20	5	3.7c	−3p
50	80	2	neg	3.5c	+7p, +8q, +12q, +13q, +16, +20q, −8p
51	20	50	neg	4.2c	+1q, +8, +20
65	10	80	80	3.3c	+20, +Xp, −1p, −4p, −5q, −17p, −18, −21
68	80	5	neg	3.5c	+1q, +5p, +6, +7, +8q, +11p, +12p, +13q, +17q, +20, +Xp, −4q, −8p, −18q
86	80	10	neg	3.8c	+2p, +7p, +8q, +17q, +20
88	60	80	neg	4.3c	+5p, +7, +13q, +20, −4, −9p, −18
89	80	80	neg	3.7c	+20

The numbers in the columns labeled MIB, DO-1, and WAF1/p21 indicate the percentages of cells positive for the respective antibody. The DNA values are expressed in relative units, with 2c reflecting a diploid DNA content in normal cells. The CGH row displays the chromosomes that are involved in copy number changes; —, no aberrations were detected.

chromosomal gains and losses in 12 high-grade adenomas analyzed in this study.

Carcinomas

Severe aneuploidy was detected in colorectal carcinomas. In many samples, the DNA histograms showed scattered values exceeding 8c. Proliferative activity was seen in more than 80% of the cells analyzed. Increased TP53 immunoreactivity was observed in most of the samples analyzed. This increase was accompanied by predominantly negative staining for p21/WAF-1 immunoreactivity.

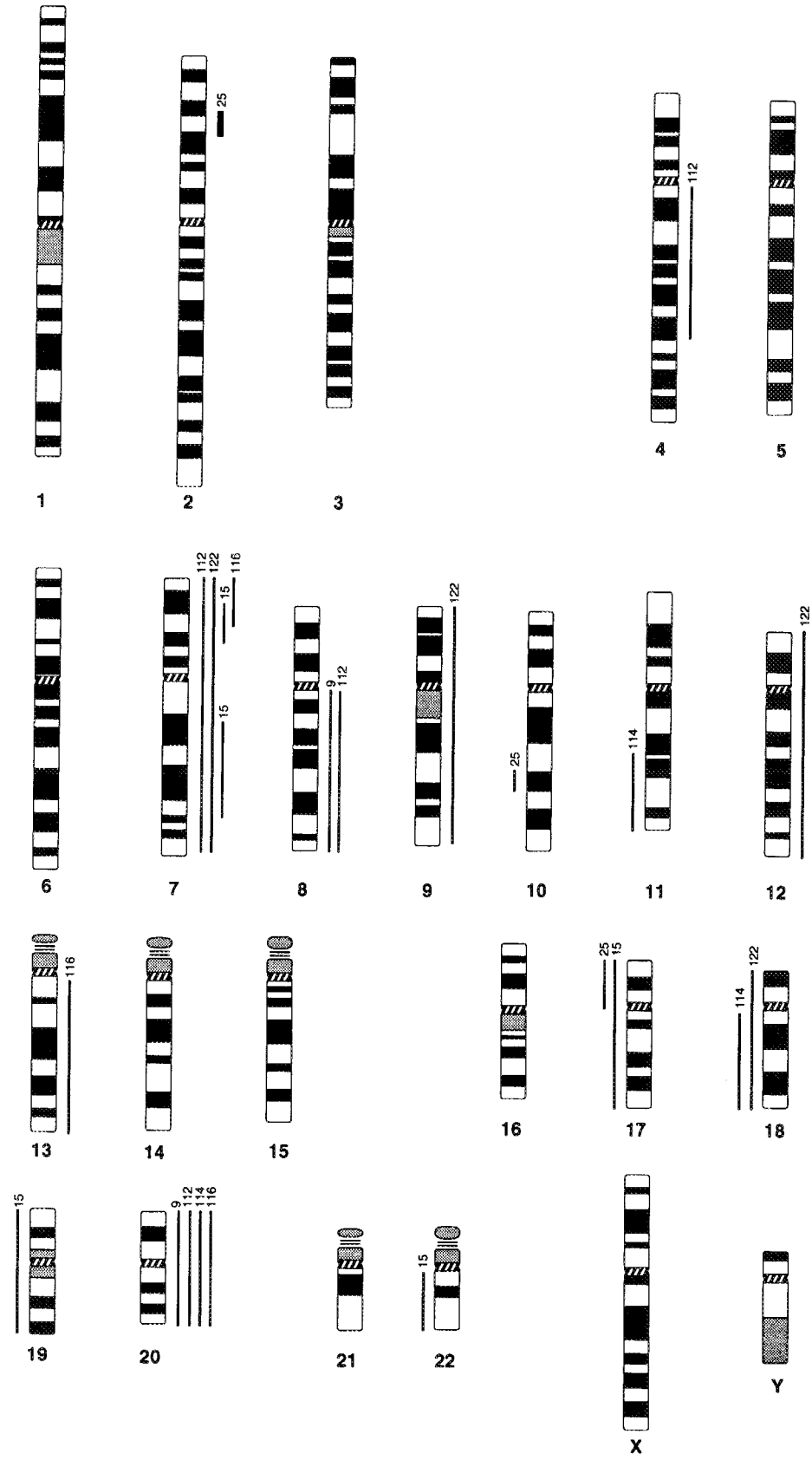

Figure 3. Summary of DNA copy number changes in high-grade adenomas (n = 12). Bars on the left side of the chromosome ideogram denote a loss, bars on the right side a gain of sequences in the tumor genome. Solid squares or bars indicate high-level copy number increases (amplifications). Individual cases can be identified by the numbers on top of the bars. Chromosomes 7 and 20 are recurrently gained in high-grade adenomas.

This indicates functionally inactive TP53 expression. (See Figure 1 for an example of the different staining patterns, the DNA histogram, and CGH in case 88 and Table 2 for a summary of the results.) The loss of functional TP53 is reflected on the genomic level by the emergence of multiple, yet specific, chromosomal aberrations. DNA copy number increases were mapped to the following chromosomal arms or chromosomes: 20q (12/16), 13q (11/16), 8q (8/16), 7p (7/16), and 1q (5/16). Decreased values were observed on chromosome arms 8p (6/16) and 18q (6/16), chromosome 4 (5/16), and chromosome arm 17p (3/16). Chromosomal arms 7p, 8q, 13q, and 20q were also subject to high-level copy number increases, whereas regional, band-specific amplifications were mapped to chromosome bands 12q13, 17q21, 18q23, and 20q13.2-13.3. In two of 16 carcinomas, no copy number changes were detected with CGH. Figure 4 summarizes the DNA copy number changes in 16 carcinomas.

DISCUSSION

Our results show that recurrent chromosomal aberrations in carcinomas of the colon are found as DNA copy number increases on chromosome arms 20q, 13q, 8q, 7p, 1q, and 5p. Decreased fluorescence values, indicating losses, were observed on chromosome 4 and chromosome arms 8p, 18q, and 17p (Fig. 4). The pattern detected in carcinomas is remarkably consistent with data from cytogenetic banding analysis. In a study of 108 cases, Bardi et al. (1995) observed consistent gains of chromosomes 7 and 13 and chromosome arm 8q as well as recurrent losses of chromosomes 4 and 18 and chromosome arms 8p and 17p. Similarly, the overrepresentation of chromosomes 7, 13, and 20 was observed in a study by Muleris et al. (1994). This correlation further corroborates the validity of CGH for screening of tumor genomes for chromosomal imbalances and for delineating complex chromosomal aberrations.

High-level copy number increases were detected in our series of carcinomas at different chromosomal locations. These copy number increases (amplifications) occurred almost exclusively at regions that were also often involved in low-level copy number increases, i.e., on 7p (which harbors the *EGFR* gene), 8q (*MYC*), 13q, and chromosome 20. This indicates that low-level copy number increases precede the high-level copy number increases (amplifications) of genes that are relevant for tumor progression. Apparently, high-level copy number increases require the loss of function of *TP53*, as indicated by elevated *TP53* expression in the absence of immunoreactivity for p21/WAF-1. Two band specific amplifications were observed on bands 12q13 and 18q23. These bands have not been described to date as being involved in gene amplifications in colon carcinomas.

Recurrent chromosomal losses were mapped to chromosome 4 and chromosome arms 8p, 17p, and 18q. Chromosome 17 harbors *TP53*, and *DCC* resides on 18q. Again, this finding is consistent with data from studies using chromosome banding and LOH (Gerdes et al., 1995). Notably, 5q was only rarely lost in our cases of sporadic colon tumors, which is in contrast to findings for familiar forms of colon cancer, in which the tumor suppressor gene *FAP* is often inactivated by deletions covering chromosomal band 5q21. Three possible explanations may account for the discrepancies: 1) The deletions on 5q are submicroscopic and thus are beyond the detection limit of CGH, 2) point mutations account for gene inactivation, or 3) loss of *APC* is less relevant for the genesis of sporadic carcinomas.

The overrepresentation of chromosome 7 occurs at early stages of colorectal tumor progression. This observation is in line with previous reports of studies using chromosome banding analysis (Bomme et al., 1994) and interphase FISH on colon adenomas (Herbergs et al., 1994). However, the relevance of the gain of chromosome 7 as a causative factor for tumor development was questioned because increases in copy numbers for chromosome 7 were also reported in nontumor tissues (Kovacs and Brusa, 1989; Elfving et al., 1992). The material analyzed in our study was microdissected from histological sections and contained mostly epithelial cells. Therefore, our findings support the evidence that gain of chromosome 7 is an early requirement for tumor development and is not due to contamination with cells of stromal origin. Also, tissue from nontumorous material did not reveal an overrepresentation of chromosome 7.

The comparison of chromosomal gains and losses in low- and high-grade adenomas and carcinomas suggests a sequence of events: It seems that the gains of chromosomes 7 and 20 as well as the losses of sequences on 17p and 18q occur relatively early, insofar as they are present in 30% and 25% of the high-grade adenomas, respectively. The gain of chromosome 13, however, is a sporadic finding in adenomas, but is the most common finding in carcinomas, where it occurs also as a high-level copy number increase (Figs. 3, 4).

A general picture of chromosomal and genomic

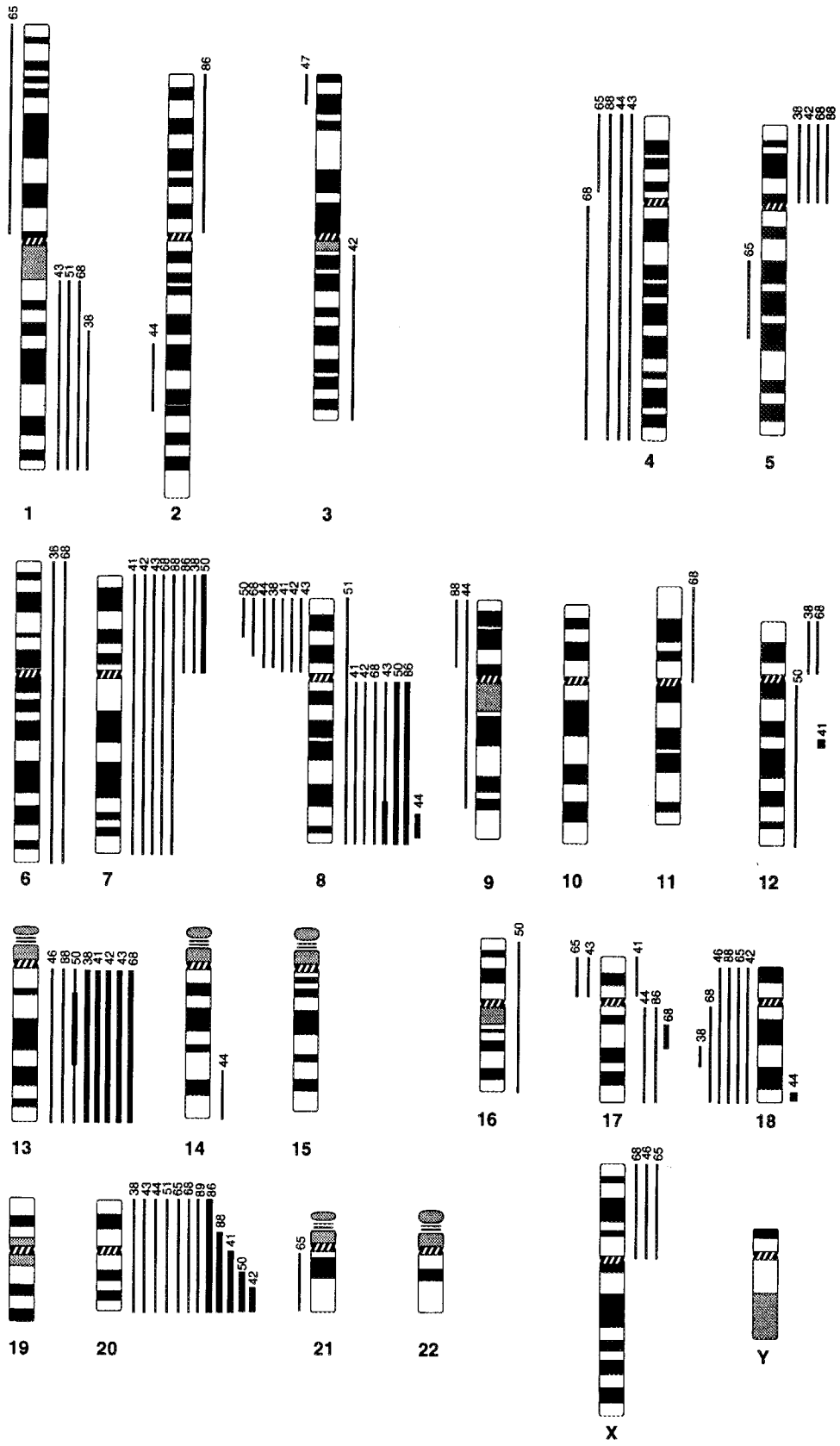

Figure 4. Summary of DNA copy number changes in carcinomas (n = 16). Bars on the left side of the chromosome ideogram denote a loss, bars on the right side a gain of sequences in the tumor genome. Solid squares or bars indicate high-level copy number increases (amplifications). Individual cases can be identified by the numbers on top of the bars. Multiple aberrations were mapped, including gains of chromosomal segments on 1q, 7p, 13q, and 20q, and losses on chromosome 4 and chromosome arms 8p, 17p, and 18q. High-level copy number increases (amplifications) are frequent.

aberrations emerges when we combine the data from our analysis: Crude aneuploidy was found already in low-grade adenomas; none of the adenomas that we analyzed displayed a diploid DNA distribution. The proliferative activity, measured by immunostaining with an antibody against Ki-67 was increased. Gain of chromosome 7 occurred in only a single case. The aneuploidy was increasing in high-grade adenomas, with a higher percentage of cells exceeding the 5c threshold. Gain of chromosomes 7 and 20 was visible in 30% of the cases. The number of nonrandom chromosomal aberrations increased significantly when the cells had progressed to a carcinoma. The gain or loss of specific chromosomal aberrations coincided with the overexpression of mutant TP53, which appeared at the transition from high-grade adenomas to carcinomas.

A study similar to that described here was performed at different stages of progression in cervical tumors (Heselmeyer et al., 1996). Similarities among colon and cervical carcinogenesis include the finding that aneuploidization precedes the loss and gain of specific chromosomes. In both tumors, the acquisition of recurrent chromosomal aberrations occurs at the transition from high-grade adenoma/severe dysplasia to invasive carcinoma. It seems that in both organs wild-type *TP53* is required to prevent the acquisition of nonrandom chromosomal aberrations that accompany the transition to invasive carcinomas. DNA indices, however, are different among cervix and colon carcinomas: The initial genetic disturbance in cervical carcinogenesis is reflected by a tetraploidization that occurs in mild and moderate dysplasias. This is in striking contrast to the severe aneuploidy that we observed in low-grade colorectal adenomas.

It should be noted that two of 16 carcinomas did not reveal any copy number changes on CGH. It is unlikely that this is due to contamination of tumor tissue with stromal cells or lymphocytic infiltrations, because we select the tumor tissue by microdissection. It is possible that a number of point mutations in protooncogenes or tumor suppressor genes due to repeat instability caused by mismatch repair deficiency is the causative mechanism in these tumors. Notably, these tumors reveal a high degree of aneuploidy as well.

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